f) further maintaining the membrane strip under conditions which allow the fluid in the sample to transport any contacted analyte-binding particles not bound to the sample capture reagent or to the control capture reagent by capillary action beyond the control capture zone;.

g) determining the amount of contacted analyte-binding particles in the sample capture zone and the amount of contacted analyte-binding particles in the control capture zone;

h) determining a corrected analyte-binding particle amount from the amount of analyte-binding particles in the sample capture zone and the amount of analyte-binding particles in the control capture zone by compensating for the amount of variability in the reaction of the analyte-binding particles with the surfaces of the assay,

wherein the amount of analyte of interest in the fluid sample is directly related to the corrected analyte-binding particle amount.

REMARKS

Rejection of Claims under 35 U.S.C. 112, second paragraph

The Examiner rejected Claims 1-15, for being indefinite. In particular, the Examiner questioned how the particles in part (c) of Claim 1 could be immobilized in the contact region, if they subsequently in step (d) migrate by capillary action. As indicated in the Specification (see, e.g., p. 12, line 9 *et seq.*; p. 14, line 1 *et seq.*) "immobilized" refers to particles that are coated on and/or permeated in the membrane. Furthermore, step (d) indicates that the fluid in the sample mobilizes and transports the contacted analyte-binding particles by capillary action. In view of these considerations, one of ordinary skill in the art would understand that the particles are coated on and/or permeated in the membrane, and that they are set into motion by the fluid which transports them by capillary action.

The Examiner also indicated that it was unclear regarding to what the control capture reagent was binding in part (e) of Claim 1. As indicated in the Specification (see, e.g., p. 14, line 11 *et seq.*), the control capture reagent reacts with the analyte binding particles, but does not

interact with the analyte to be measured: for example, the control capture reagent can react with the analyte-binding agent on the analyte-binding agent-coated particles; with another material on the particles; or with the particles themselves.

Part (e) of Claim 1 indicates that "contacted analyte-binding particles" bind to the control capture reagent. "Contacted analyte-binding particles," as indicated in the Specification (see, e.g., p. 16, line 3 *et seq.*), are analyte-binding particles which have been maintained under conditions allowing analyte in the fluid (if present) to bind to the analyte-binding particles immobilized in the contact region. Thus, contacted analyte-binding particles may or may not have analyte bound to the analyte-binding agent, depending on whether or not analyte is present in the fluid sample and whether analyte has bound to the analyte-binding agent on the analyte-binding particles. Because there are multiple binding sites for analyte on the analyte-binding particles, the presence and the concentration of analyte bound to analyte-binding particles varies; the concentration of analyte bound to the analyte-binding particles increases proportionally with the amount of analyte present in the fluid sample, and the probability of an analyte-binding particle being arrested in the sample capture zone (as described below) similarly increases with increasing amount of analyte bound to the analyte-binding particles. Thus, the population of contacted analyte-binding particles may comprise particles having various amount of analyte bound to the analyte-binding agent, as well as particles having no analyte bound to the analyte-binding agent (just as the analyte-binding particles initially have no analyte bound to the analyte-binding agent).

In view of these considerations, one of ordinary skill in the art would understand that the control capture reagent binds to contacted analyte-binding particles which may bind to the analyte-binding agent on the particles, with other material on the particles, or with the particles themselves, regardless of whether analyte is bound to the analyte-binding agent on the particles.

The Examiner also indicated that it was unclear how the amount of corrected analyte-binding particles was determined. As described in detail in the Specification (see, e.g., p. 7, line 11 *et seq.)* the methods of the invention involve inclusion, within the assay, of an internal control that includes a control capture reagent that specifically binds to analyte-binding particles. The behavior of the analyte-binding particles with regard to the control capture reagent is used to compensate for the amount of variability in the reaction of the analyte-binding particles with the

surfaces of the assay, and the amount of variability of the analyte-binding particles can be taken into consideration in a determination of the amount of analyte of interest. In some embodiments, for example, a corrected amount of analyte-binding particles can be determined by use of a ratio between 1) the amount of analyte-binding particles that are arrested in the sample capture zone, and 2) the amount of analyte-binding particles in the control capture zone; or use of a ratio between 1) the amount of analyte-binding particles that are arrested in the sample capture zone, and 2) the sum of the amount of analyte-binding particles in the control capture zone and the amount of analyte-binding particles that are arrested in the sample capture zone. Other appropriate calculations can also be used to eliminate the variability in the specific binding component of the reaction. The amount of analyte of interest can then be calculated from the corrected amount of analyte-binding particles.

Step (h) has therefore been amended to indicate that the a corrected analyte-binding particle amount is determined by compensating for the amount of variability in the reaction of the analyte-binding particles with the surfaces of the assay. In view of these considerations, one of ordinary skill in the art would understand that the corrected analyte-binding particle amount could be calculated by using the amount of analyte-binding particles in the sample capture zone and the amount of analyte-binding particles in the control capture zone to compensate for variability as described

The Examiner also stated that it was unclear how particles were labeled in Claim 6, and states that "From claim 1, it appears that the particles are the label." Applicants respectfully request clarification of this rejection. The particles described in Claim 1 are integral parts of the assay, and more than solely "labels" for different components of the assay. The Specification describes representative particle labels, for example, at p. 12, line 26 *et seq.*. The Specification states that the particles are labeled by a means which does not significantly affect the physical properties of the particles; for example, the particles are labeled internally (that is, the label is included within the particle, such as within the liposome or inside the polystyrene latex bead). The Specification further indicates that representative labels include luminescent labels; chemiluminescent labels; phosphorescent labels; enzyme-linked labels; and colorimetric labels, such as dyes or fluorescent labels. In view of these considerations, one of ordinary skill in the art would understand what is encompassed by the term "label" with regard to the particles.

Rejection of Claims under 35 U.S.C. 102(e)

The Examiner rejected Claims 1-15 under 35 U.S.C. 102(e) as being anticipated by Kuo *et al.* (US Patent 6,436,721).

Applicants' Attorney notes that the Examiner stated that the changes made to 35 U.S.C. 102(e) by the American Inventors Protection Act of 1999 (AIPA) do not apply to the examination of this application as the application being examined was "not... filed on or after November 29, 2000" or voluntarily published under 35 U.S.C. 122(b). It should be noted, however, that the current application was filed on March 26, 2001, which is after November 29, 2000. Thus, it should be examined under 35 U.S.C. after the amendment by the AIPA.

In order for a reference to anticipate claims, the reference must teach every aspect of the claimed invention either explicitly or impliedly (see M.P.E.P. § 2131).

Kuo *et al.* describe an immunochromatographic assay with a contact zone (the first zone) containing a labeled analyte binding partner that binds to the analyte of interest; a second zone that contains either immobilized analyte or immobilized antibody to the analyte (provided that it is specific for an epitope of the analyte that is different from that to which the binding partner binds); a third zone which contains a means for capturing analyte/labeled specific binding partner complex; and a fourth zone that measures a second analyte (e.g., creatinine in urine) as an indication of the concentration of the test sample.

If the second zone of Kuo *et al.* contains immobilized binding partner (e.g., antibody) to the analyte of interest, Kuo *et al.* specify that the immobilized binding partner must be specific for an epitope of the analyte that is different from the epitope to which the labeled binding partner is specific. However, the present invention contemplates that the sample capture reagent can be directed against the same epitope of the analyte as, or against a different epitope of the analyte from, the epitope that binds to the antibodies used as analyte-binding agents coated on the particles.

Kuo *et al.* state that the second zone can also contain immobilized analyte. The present invention does not contemplate utilizing immobilized analyte. Rather, the "sample capture reagent" of the present invention is a reagent that forms a binding pair with the analyte of interest, in that it specifically and preferentially binds to the analyte of interest. Furthermore, if the second zone of Kuo *et al.* contains immobilized analyte, then when the labeled analyte

binding partner mixes with analyte in the sample, excess non-bound binding partner (i.e., without analyte attached) bind in the second zone. Thus, both unbound material and bound material are measured in the second zone. The second zone will be inversely proportional to analyte concentration in the sample, and the amount of analyte/labeled specific binding partner in the third zone will be directly proportional to the analyte concentration. The ratio of the signals of the second and third zones increase the sensitivity of the assay, but because both the signals in the second zone and the third zone are measuring antigen concentration, they do not compensate for assay variability in the reaction of analyte binding particles with the surfaces of the membrane strip, as is performed in the current invention.

The concentration of the separate marker (in the fourth zone) is used in a ratio to correct for the concentration of the analyte of interest in the test sample in Kuo *et al.* The present invention does not utilize such a separate marker or zone. Furthermore, the separate marker of Kuo *et al.* is not used for correcting assay variability, and, in fact, cannot be used to correct for assay variability since the concentration of the separate marker is unknown. In contrast to the methods described by Kuo *et al.*, the methods of the invention utilize an internal control that is a known quantity: therefore any variation of its signal is due to assay variability.

In view of these considerations, Kuo *et al.* do not teach every aspect of the claimed invention. Therefore, the claimed invention is not anticipated by Kuo *et al.*

CONCLUSION

In view the amendments and discussion presented above, the application in condition for allowance. Applicants' Attorney respectfully requests that the Examiner reconsider and withdraw all rejections.

If the Examiner believes that a telephone conversation would expedite prosecution of the application, the Examiner is invited to call Elizabeth W. Mata at (915) 845-3558 (Mountain time

zone). If Elizabeth W. Mata cannot be reached, the Examiner is invited to call David E. Brook at (978) 341-0036.

Respectfully submitted,

HAMILTON, BROOK, SMITH & REYNOLDS, P.C.

David E. Brook, R.N. 22592

By for Elizabeth W. Mata

Elizabeth W. Mata
Registration No. 38,236
Telephone: (978) 341-0036
Facsimile: (978) 341-0136

Concord, MA 01742-9133

Dated: 12/18/02

MARKED UP VERSION OF AMENDMENTS

Claim Amendments Under 37 C.F.R. § 1.121(c)(1)(ii)

1. (Amended) A method for quantitatively measuring the amount of an analyte of interest in a fluid sample, comprising:

a) providing a membrane strip comprising an application point, a contact region, a sample capture zone and a control capture zone, wherein the contact region is between the application point and the sample capture zone and the sample capture zone is between the contact region and the control capture zone;

b) contacting the application point of the membrane strip with the fluid sample to be assayed for the analyte of interest;

c) maintaining the membrane strip under conditions which allow fluid to transport analyte of interest in the fluid sample by capillary action through the strip to and through the contact region, the contact region having a population of analyte-binding particles immobilized therein, wherein the analyte-binding particles are coated with an analyte-binding agent;

d) further maintaining the membrane strip under conditions which allow analyte of interest, if present in the sample, to bind to analyte-binding particles, thereby generating contacted analyte-binding particles; allow the fluid in the sample to mobilize and transport contacted analyte-binding particles by capillary action through the strip to and through the sample capture zone, the sample capture zone having a sample capture reagent immobilized thereon; and allow contacted analyte-binding particles to bind to the sample capture reagent;

e) further maintaining the membrane strip under conditions which allow the fluid in the sample to transport contacted analyte-binding particles by capillary action through the strip to and through the control capture zone, the control capture zone having a control capture reagent immobilized thereon; and allow contacted analyte-binding particles to bind to the control capture reagent;

f) further maintaining the membrane strip under conditions which allow the fluid in the sample to transport any contacted analyte-binding particles not bound to the sample capture reagent or to the control capture reagent by capillary action beyond the control capture zone;.

g) determining the amount of contacted analyte-binding particles in the sample capture zone and the amount of contacted analyte-binding particles in the control capture zone;

h) determining a corrected analyte-binding particle amount from the amount of analyte-binding particles in the sample capture zone and the amount of analyte-binding particles in the control capture zone by compensating for the amount of variability in the reaction of the analyte-binding particles with the surfaces of the membrane strip,

wherein the amount of analyte of interest in the fluid sample is directly related to the corrected analyte-binding particle amount.